

# Certificate of Analysis

## National Institutes of Health

BLDG 14A, RM 119A8

14 Service Rd West

Bethesda Maryland 20892-5515 United States

|                            |                              |                          |                              |
|----------------------------|------------------------------|--------------------------|------------------------------|
| <b>Sample Name:</b>        | <b>NIH31-Z031811</b>         | <b>Covance Sample:</b>   | <b>801667</b>                |
| <b>Project ID</b>          | NAT_INST-20110504-0017       | <b>Receipt Date</b>      | 04-May-2011                  |
| <b>PO Number</b>           | Req #2124713 / Charge - Visa | <b>Receipt Condition</b> | Ambient temperature          |
| <b>Sample Serving Size</b> |                              | <b>Login Date</b>        | 04-May-2011                  |
|                            |                              | <b>Storage Condition</b> | -20 (+/- 10) Degrees Celsius |

| Analysis/Result                                | Result        |
|--|---------------|
| <b>Fatty Acids calculated as Triglycerides</b> |               |
| 8:0 Caprylic                                   | <0.005 g/100g |
| 10:0 Capric                                    | <0.005 g/100g |
| 12:0 Lauric                                    | <0.005 g/100g |
| 14:0 Myristic                                  | 0.078 g/100g  |
| 14:1 Myristoleic                               | <0.005 g/100g |
| 15:0 Pentadecanoic                             | 0.011 g/100g  |
| 15:1 Pentadecenoic                             | <0.005 g/100g |
| 16:0 Palmitic                                  | 0.909 g/100g  |
| 16:1 Palmitoleic                               | 0.096 g/100g  |
| 17:0 Heptadecanoic                             | 0.013 g/100g  |
| 17:1 Heptadecenoic                             | <0.005 g/100g |
| 18:0 Stearic                                   | 0.199 g/100g  |
| 18:1 Oleic                                     | 1.14 g/100g   |
| Total 18:1 Isomers                             | 1.23 %        |
| 18:2 Linoleic                                  | 2.46 g/100g   |
| 18:3 Gamma Linolenic                           | <0.005 g/100g |
| 18:3 Linolenic                                 | 0.215 g/100g  |
| 18:4 Octadecatetraenoic                        | 0.022 g/100g  |
| 20:0 Arachidic                                 | 0.020 g/100g  |
| 20:1 Eicosenoic                                | 0.028 g/100g  |
| 20:2 Eicosadienoic                             | 0.006 g/100g  |
| 20:4 Arachidonic                               | 0.013 g/100g  |
| 20:3 Eicosatrienoic                            | <0.005 g/100g |
| 20:5 Eicosapentaenoic                          | 0.110 g/100g  |
| 22:0 Behenic                                   | 0.017 g/100g  |
| 22:1 Erucic                                    | 0.006 g/100g  |
| 22:5 Docosapentaenoic                          | 0.023 g/100g  |
| 24:0 Lignoceric                                | 0.012 g/100g  |
| 22:6 Docosahexaenoic                           | 0.145 g/100g  |
| Saturated Fat                                  | 1.20 g/100g   |
| Monounsaturated Fat, Cis and Trans Isomers     | 1.31 g/100g   |
| Polyunsaturated Fat, Cis and Trans Isomers     | 2.87 g/100g   |
| Omega 3 Fatty Acids                            | 0.515 g/100g  |
| Omega 6 Fatty Acids                            | 2.48 g/100g   |



Report Number: 386359-0

Report Date: 13-May-2011

Report Status: Final

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|                            |                              | <b>Storage Condition</b> | -20 (+/- 10) Degrees Celsius |

| Analysis/Result                                | Result        |
|--|---------------|
| <b>Fatty Acids calculated as Triglycerides</b> |               |
| Sum of Fatty Acids                             | 5.62 g/100g   |
| <b>Isoflavone</b>                              |               |
| Daidzin  | 6.11 mg/100g  |
| Glycitin                                       | 8.53 mg/100g  |
| Genistin                                       | 9.17 mg/100g  |
| Daidzein                                       | <1.00 mg/100g |
| Glycitein                                      | <1.00 mg/100g |
| Genistein                                      | <1.00 mg/100g |
| Total as Glucosides                            | 23.8 mg/100g  |

| Method References | Testing Location |
|-------------------|------------------|
|-------------------|------------------|

**Fatty Acids calculated as Triglycerides (FALC\_S:10)** Covance Laboratories Inc.

Official Methods and Recommended Practices of the AOCS, Official methods Ce 1-62 (1997), Ce 2-66, Ce 1d-91, Ce 1k-07 (2007), and Ce 1i-07 (2007), The American Oil Chemists' Society, Champaign, IL (modified).

**Isoflavone (ASOF\_S:3)** Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, (2005) 18th ED., AOAC INTERNATIONAL Gaithersburg, MD, USA, Official Methods 2001.10. (Modified).

| Testing Location(s) | Released on Behalf of Covance by |
|---------------------|----------------------------------|
|---------------------|----------------------------------|

**Covance Laboratories Inc.** Lori Ross - Associate Director

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Madison WI 53704  
608-242-2712 x4170

These results apply only to the items tested. This certificate of analysis shall not be reproduced, except in its entirety, without the written approval of Covance.

**NIH SPECIFICATION****Open Formula Rat and Mouse Diet - 18%  
Crude Protein Autoclavable (NIH-31)**

| <u>Ingredients</u>                              | <u>Percentage by Weight</u> |
|---|-----------------------------|
| Fish meal (60% protein)                         | 9.0                         |
| Soybean meal (48.5% protein)                    | 5.0                         |
| Alfalfa meal (17% protein)                      | 2.0                         |
| Corn gluten meal (60% protein)                  | 2.0                         |
| Ground whole hard wheat                         | 35.5                        |
| Ground #2 yellow shelled corn<br>(8.5% protein) | 21.0                        |
| Ground whole oats                               | 10.0                        |
| Wheat middlings                                 | 10.0                        |
| Brewers dried yeast                             | 1.0                         |
| Soy oil   | 1.5                         |
| Salt  | .5                          |
| Dicalcium phosphate                             | 1.5                         |
| Ground limestone                                | .5                          |
| Premixes  | .5                          |
|   | <u>100.0</u>                |

**Vitamin Fortification per ton (2,000 lbs) of Finished  
Product.**

| <u>Vitamin</u>                 | <u>Amount</u> | <u>Source</u>               |
|--------------------------------|---------------|-----------------------------|
| A                              | 22,000,000 IU | Vitamin A                   |
| Palmitate or<br>Acetate        |               |                             |
| D <sub>3</sub>                 | 3,800,000 IU  | D activated                 |
| animal sterol                  |               |                             |
| K                              | 20 g.         | Menadione                   |
| dl Alpha-tocopheryl<br>acetate | 15 g.         |                             |
| Choline                        | 700 g.        | Choline<br>chloride         |
| Folic acid                     | 1 g.          |                             |
| Niacin                         | 40 g.         |                             |
| d Pantothenic acid             | 25 g.         | d-Calcium<br>Pantothenate   |
| Riboflavin supplement          | 5 g.          |                             |
| Thiamin                        | 65 g.         | Thiamin mono<br>nitrate     |
| B <sub>12</sub> supplement     | 40,000 mcg.   |                             |
| Pyridoxine                     | 5 g.          | Pyridoxine<br>hydrochloride |
| Biotin                         | 120 mg.       | d-Biotin                    |

**Mineral Fortification per ton (2,000 lbs.) of Finished Product**

| <u>Mineral</u> | <u>Amount</u> | <u>Source</u>    |
|----------------|---------------|------------------|
| Cobalt         | 400 mg.       | Cobalt carbonate |
| Copper         | 4 g.          | Copper sulfate   |
| Iron           | 60 g.         | Iron sulfate     |
| Magnesium      | 400 g.        | Magnesium oxide  |
| Manganese      | 100 g.        | Manganese oxide  |
| Zinc           | 10 g.         | Zinc oxide       |
| Iodine         | 1500 mg.      | Calcium iodate   |

These concentrations of vitamins and minerals shall be added to the ration via two separate (vitamin and mineral) premixes. The final formulation may be adjusted so the total amount of ingredients will equal 100%. In the case of the mineral fortification, the actual amount of each element required is specified. Therefore, the contractor shall adjust the amount of each compound used in the premix according to its mineral concentration.

**Nutrient Standards**

Micro Analysis - The total calculated concentrations of nutrients in the ration from ingredients and from the fortifications at the time of manufacture should be as follows:

|               |   |         |      |
|---------------|---|---------|------|
| Crude protein | % | Minimum | 18.0 |
| Crude fat     | % | Minimum | 4.0  |
| Crude fiber   | % | Maximum | 5.0  |
| Ash           | % | Maximum | 8.0  |

**Amino Acids (% of total diet)**

|               |         |
|---------------|---------|
|               | Minimum |
| Arginine      | .90     |
| Lysine        | .85     |
| Methionine    | .35     |
| Cystine       | .25     |
| Tryptophan    | .20     |
| Glycine       | .95     |
| Histidine     | .38     |
| Leucine       | 1.40    |
| Isoleucine    | .95     |
| Phenylalanine | .85     |
| Tyrosine      | .60     |
| Threonine     | .65     |
| Valine        | .90     |

**Minerals**

|             |     |         |        |
|-------------|-----|---------|--------|
| Calcium     | %   | Minimum | 1.15   |
| Phosphorous | %   | "       | .85    |
| Potassium   | %   | "       | .75    |
| Sodium      | %   | "       | .30    |
| Magnesium   | %   | "       | .15    |
| Iron        | PPM | "       | 345.00 |
| Zinc        | PPM | "       | 40.00  |
| Manganese   | PPM | "       | 140.00 |
| Copper      | PPM | "       | 12.00  |
| Cobalt      | PPM | "       | 0.70   |
| Iodine      | PPM | "       | 1.80   |

### **Vitamins**

|                         |        |   |             |
|-------------------------|--------|---|-------------|
| Vitamin A               | IU/g   | " | 20.0 (10) * |
| Vitamin D               | IU/g   | " | 4.0         |
| Alpha-tocopherol        | PPM    | " | 45.0        |
| Thiamin                 | PPM    | " | 70.0        |
| Riboflavin              | PPM    | " | 7.0         |
| Niacin                  | PPM    | " | 80.0        |
| Pantothenic Acid        | PPM    | " | 30.0        |
| Choline                 | PPM    | " | 1900.0      |
| Pyridoxine              | PPM    | " | 10.0        |
| Folic Acid              | PPM    | " | 2.0         |
| Biotin                  | PPM    | " | .2          |
| Vitamin B <sub>12</sub> | mcg/kg | " | 40.0        |
| Vitamin K               | PPM    | " | 20.0        |

\* TRUE VITAMIN A ACTIVITY BY HPLC METHOD